Bioremediation and Revegetation to restore the public use of contaminated land

LIFE15 ENV/IT/000396

Life BIOREST Project
LAYMAN'S report

Project Title: "Bioremediation and Revegetation to restore the public use of contaminated land"
Abbreviation: Life BIOREST
Reference: LIFE 15ENV/IT/000396

Coordinating beneficiary: Consorzio Italbiotec

Associated beneficiaries: Actygea Srl, Agenzia regionale per la Prevenzione, l'Ambiente e l'Energia - ARPAE, University of Turin, Università Cattolica del Sacro Cuore, Agencia Estatal Consjeio Superior de Investigaciones Cientificas (Spain) and Welience - SATT - SAYENS (France)

Total budget: € 1.710.267, EU Co-financing: € 968.274
Period: 1 July 2016 to 31 December 2019

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Introduction

As a non-renewable resource, soil needs to be protected and managed in a sustainable way. It's true that in some cases, soil degradation is a natural phenomenon, but anthropized activities exacerbate it. Soil pollution represents a direct consequence of the industrial activities and improper disposal of waste, with a detrimental effect on the ecosystem services provided by soil.
Soil pollution reduces food security, negatively affecting crop yields and making them unsafe for the animal and human consumption.
Many contaminants are transported from the soil to surface waters and groundwater, causing enormous environmental harm and direct human health issues.
Furthermore, pollutants directly stress soil macro and microorganisms, affecting soil biodiversity.
In the European Union, 42 potentially contaminated sites and 5.7 contaminated sites per 10,000 inhabitants are present, with about 340,000 sites that require remediation.
Currently, only about 15% of contaminated soils have been subjected to clean-up operations. The most common soil pollutants in Europe are heavy metals, followed by mineral oil, polycyclic aromatic hydrocarbons (PAHs) and mixtures of benzene, toluene, ethylbenzene and xylene (BTEX). The contamination of air, water and soil by these compounds represents a threat to the health and the environment that needs to be resolved efficiently.

Bioremediation: the use of nature to fix nature

Currently, the main techniques used for remediating polluted soils include thermal, chemical and physical methods. They often have technical and economic drawbacks such as transferring the pollution to a new phase (liquid or air), drastically changing soil properties, including biodiversity. They are also not adequate to treat large volumes since they are generally costly.

Bioremediation represents a cost-effective alternative to the traditional remediation approaches, which uses microorganisms, such as fungi and bacteria, to degrade hazardous substances present in the polluted soil, by enhancing the natural biodegradation process.
Even though bioremediation is considered an effective technique, its diffusion is still very limited: in Europe, it is applied in less than 20% of cases.
For this reason, LIFE BIOREST project proposes a model of bioremediation of a polluted hydrocarbon site, which aims to demonstrate the effectiveness and the economic sustainability of this approach, by taking into account the role of biodiversity and embracing the concept of the circular and green economy.

The project partners

The LIFE BIOREST project arises from the experience of its European partnership, coordinated by Consorzio Italbiotec, the first Italian no-profit organization for the biotechnological development, and the associated beneficiaries Actygea Srl, Agenzia regionale dell'Emilia-Romagna per la Prevenzione, l'Ambiente e l'Energia – ARPAE, University of Turin, Università Cattolica del Sacro Cuore, CSIC (Spain) and SATT-SAYENS (France).

Life BIOREST: a model of urban regeneration

The LIFE BIOREST project aims at validating a sustainable bioremediation model for soil polluted by PAHs, BTEX and long-chain alkanes, based on the use of bacteria and fungi naturally present in the polluted soil.
The project focuses on the bioremediation of a portion of the well-characterized National Interest Site (SIN) of Fidenza, Italy. Remediation activities based on biopile were already active in this site, but they did not include bioaugmentation with selected microorganisms.
Therefore, LIFE-BIOREST adopts an integrated approach based on bioaugmentation with autochthonous and ecologically adapted bacteria and fungi, use of local agro-food by-products and revegetation, leading to lower residual pollutant concentrations to re-vegetate and restore the contaminated soil and its return to public use.
One of the main goals of the Life BIOREST project is to demonstrate the feasibility of the scale-up at the industrial level of the production of microorganisms and, for this reason, it is strongly committed to select the most suitable microbial consortia.
LIFE BIOREST uses agro-food by-products such as rice husks as a carrier for the microbial biomass into the biopile and biostimulant for the activity of degrading microbes. This results in a valorisation of by-products that are widely present all around the EU. The successful demonstration of eco-friendly bioremediation techniques in an important national interest site has the potential to trigger new business models to address a problem that is still mostly unsolved in Europe.

The area of the project

Experimental activities started in July 2016, in the area of "ex-Carbochimica", Site of National Interest (SIN) in Fidenza, thanks to the support of the Municipality that provided infrastructure and spaces already affected by other reclamation activities.
The industrial pole of "ex-Carbochimica" and "ex-CIP" (two companies active there from 1888 to 2003), has an extension of 115,000 square meters. In 2001 the Municipality of Fidenza started the reclamation of the "ex-Cip" site and in 2013 of the "ex-Carbochimica site".

The characterization of the pollutants has highlighted a situation of worrying contamination: chlorinated solvents (chloroform, perchloroethylene, carbon tetrachloride), phenols, heavy and light hydrocarbons, PAH (naphthalene, anthracene), BTEX and aliphatic chlorinated solvents at very high concentrations. The site is located just less than 1 km from the city centre and has been impacted for decades by several industries. They were relevant contributors to the local economic growth but were responsible for negative health effects and chemical pollution.

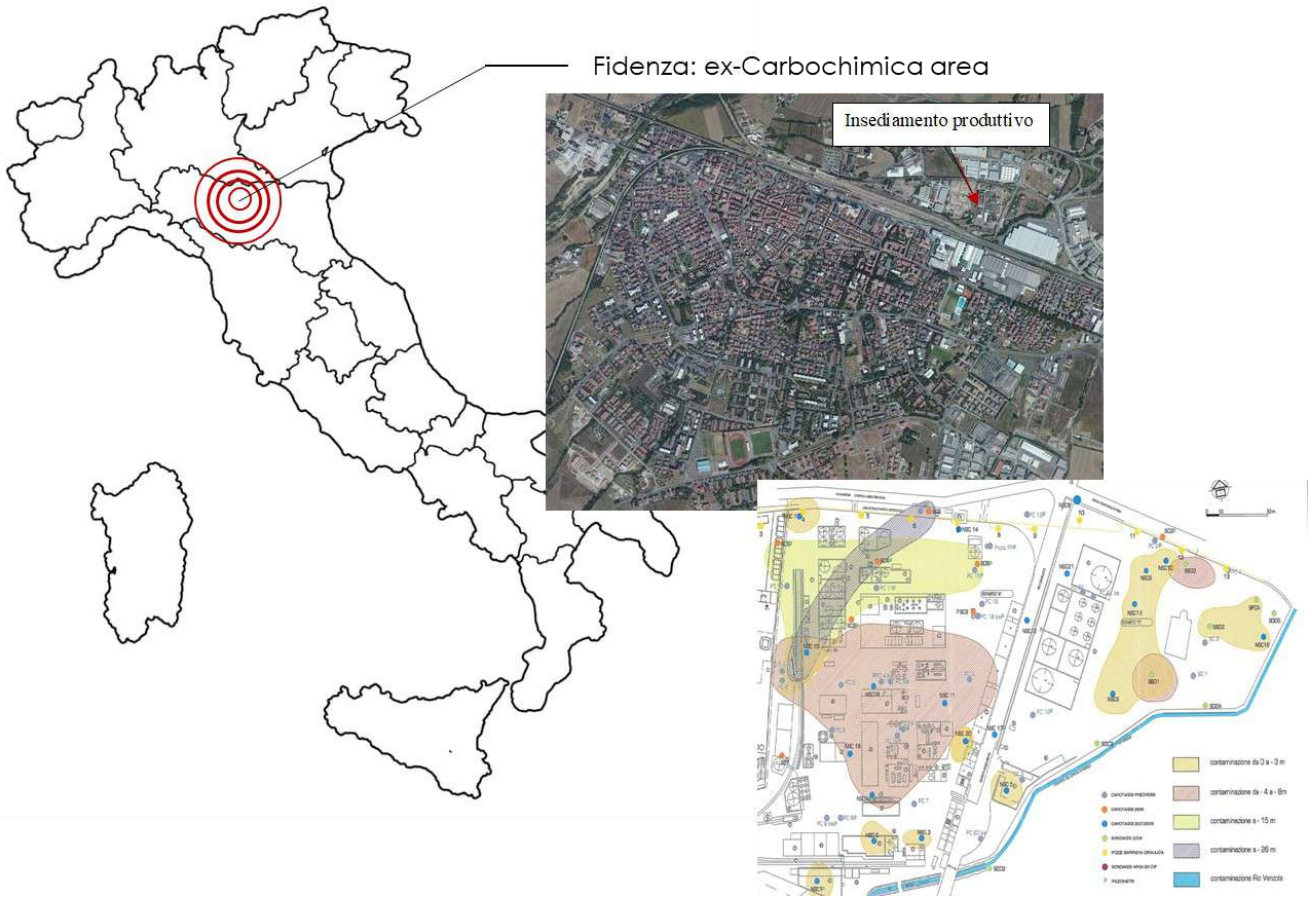


Figure 1. ex-Carbochimica area, Site of National Interest (SIN) in Fidenza

Objectives

- *Demonstrate the efficiency and the economic sustainability of a bioremediation approach for soils polluted by hydrocarbons, based on the bioaugmentation of bacteria and fungi, and the utilization of local agro-food by-products;*
- *Demonstrate the feasibility of the scale-up, at the industrial level, of the production of microorganisms active in bioremediation;*
- *Revegetate the decontaminated soil, restoring its ecological functions, and returning it to public use;*
- *Optimize bioremediation protocols and guidelines that can be successfully applied to other scenarios at the regional, national and European context;*
- *Spread the knowledge and consciousness about environmental pollution, risk assessment, biodiversity, ecotoxicology, soil ecology, and bioeconomy;*
- *Disseminate at the European level the societal benefits of soil contamination.*

Actions

Optimization of soil bioremediation by the selection of microbial strains with high pollutant degradation capacity, selection of the most indicated plants for phytoremediation and valorisation of agro-food by-products:

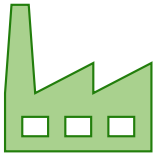
The first phase of the project is aimed at selecting a library of bacteria and fungi (strains) "with high pollutant degradation capacity" by a screening process. Individual strains or selected microbial consortia are produced in the volumes required for experimental tests (microcosms and mesocosms) and or for field application.

Microcosm:

Limited controlled environment, which reflects a wider reality

Mesocosm:

Higher-scale experimental system that examines the natural environment under controlled conditions. The mesocosm studies provide a link between field investigations and highly controlled laboratory experiments



Scale-up industrial production of microorganisms

After the analysis of the biodegradation results collected in the microcosm and mesocosm experiments, the second phase of the project is aimed at the creation of a microorganism production protocol at the industrial scale (bioaugmentation) for the remediation of *ca.* 400 m³ of soil. To make the entire process economically convenient and effective, it is also provided with the culture conditions, optimization and a production costs analysis.

Bioremediation in situ and revegetation

The third phase of the project is aimed at the treatment of the target site by "biopile". The soil treatment provides the application of bioaugmented microorganisms (a mixture of selected strains). The soil revegetation is aimed to restore proper ecological functions. Revegetation of the area is performed by selecting ecologically adapted plant species with proven degradation abilities towards the target pollutants.

Results

Optimized soil bioremediation by selected degrading strains and valorisation of agro-food by-products

Isolation and catalytic screening of bacteria and fungi

- A total of 565 microorganisms (309 fungi and 256 bacteria) were isolated from the contaminated soil and were evaluated for their performance against the pollutants of interest and
- To have an insight about their metabolic pathways, the isolated strains have been tested for their ability to produce biosurfactants and oxidative enzymes. The 70% of strains showed a positive response on at least one performed test (drop collapsing assay, blue agar test, emulsification activity, oil dispersion test). Thirty-one bacteria and 30 fungi gave a clear halo of crude oil clarification, as wide as a synthetic surfactant (*e.g.* Tween 80), indicating the presence of biosurfactants in the liquid culture. Thirty-five % of fungi clearly produced extracellular laccases, whereas only 3 Basidiomycetes showed a strong peroxidase activity.

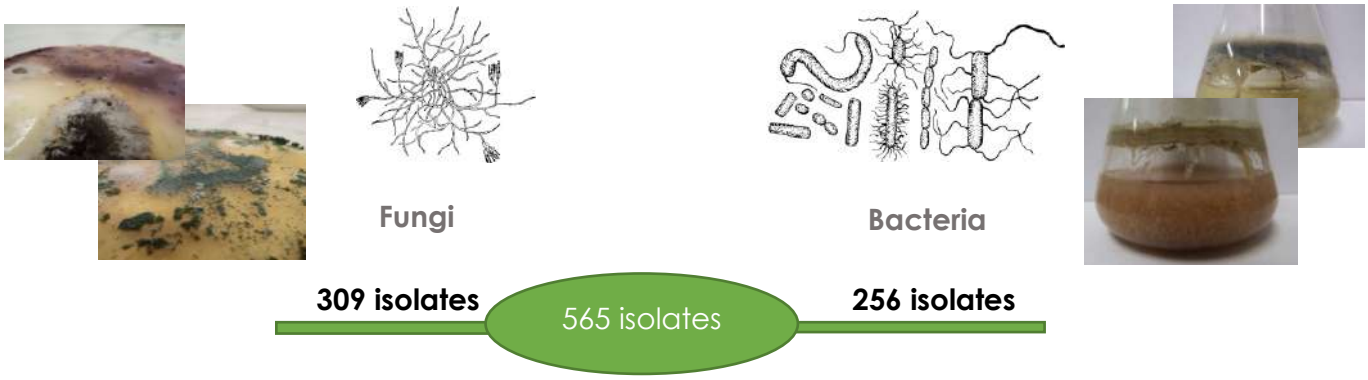


Figure 2. Aspergillus terreus

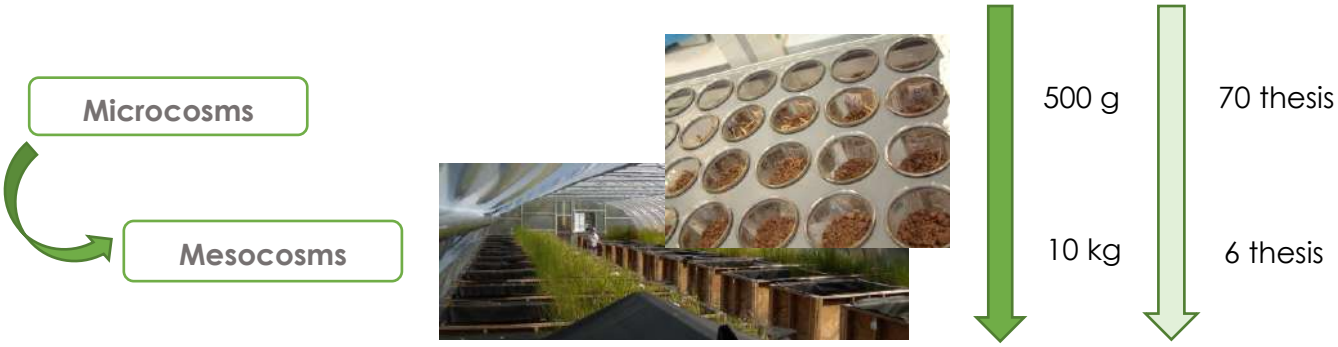
Optimization of bioremediation protocols

- Strains were then selected according to different parameters, such as their ability to grow in the presence of pollutants as sole C source, remarkable biosurfactant production and the capability to colonize lignocellulosic carriers.

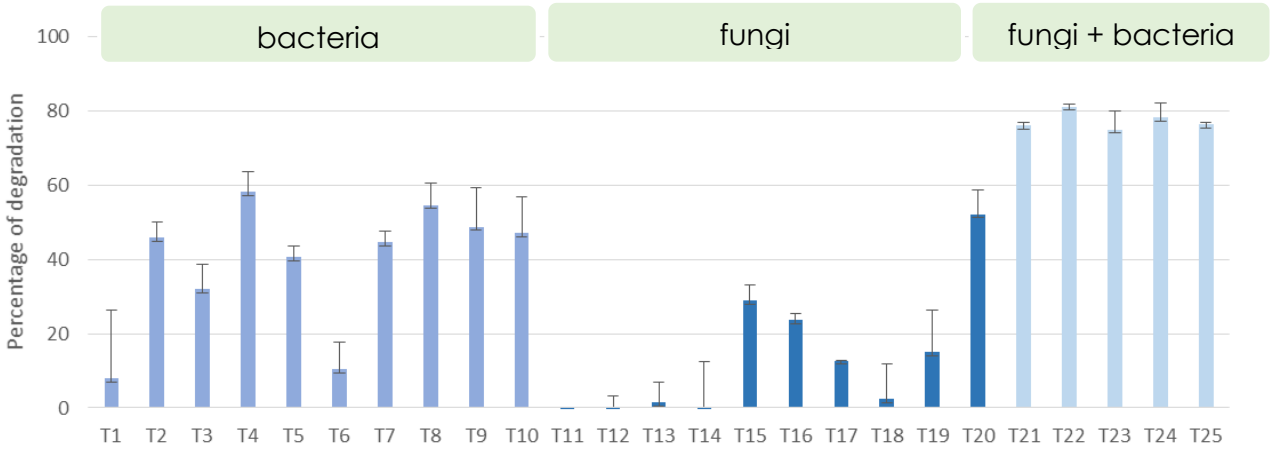
- The possibility to use agro-waste carriers as direct support to prepare the microbial inoculum was evaluated. Among others, rice husk, solid digestate, chipped wood, cornflour and grain caryopsis were considered, and rice husk was the most proper agro-food waste to be applied in the site of interest for the bio-augmented microbial consortium here proposed.
  The substrates should provide enhancement of the soil aeration, supporting fungal and bacterial biomass.

Figure 3. Rice husk

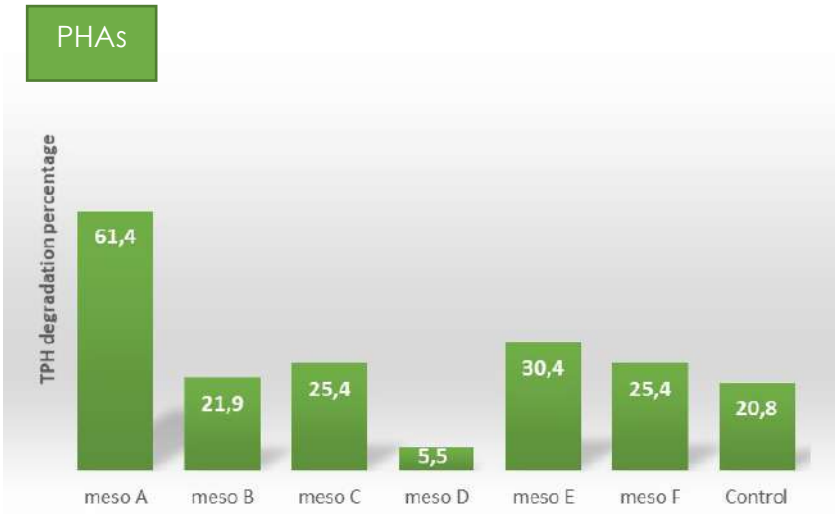
- Selected consortia were inoculated in microcosms and mesocosms trials, upscaling the treated soil from 500 g up to 10 kg.



- Bacteria and fungi were first tested separately in microcosms made up of single or mixed strains. The microorganisms were then combined, inoculating up to 35 microcosms per trial.
  Microcosm results. The graph reports the percentage of degradation of pyrene (an aromatic polycyclic hydrocarbon): the combination of fungi and bacteria consortia were faster and more efficient than separated bacteria and fungi.



- Six different microbial consortia were inoculated in mesocosm trials.
  Mesocosm results. In the optimal conditions, total hydrocarbon content decreased up to 70%, with main differences among the different thesis, as can be seen in the graph below. In the best performing consortium, the most concentrated pollutants (*e.g.* phenanthrene, fluoranthene and pyrene) were almost completely transformed.



- According to the chemical, ecotoxicological and biological monitoring, the consortium made up of *Fusarium solani, Talaromyces sayulitensis, Aspergillus jensenii, Aspergillus terreus, Trametes gibbosa* and *Bjerkandera adusta* among fungi, and of *Pseudomonas spp, Pseudomonas putida* and *Acinetobacter calcoaceticus* among bacteria, gave the best results in terms of degradation.

- The treated soils of all the mesocosms were then used for plant treatment.

Scale-up of the industrial production of microorganisms

Biostimulation and optimization of growth conditions

- The isolated microorganisms were tested in microcosm experiments to achieve suitable conditions for the growth to be applied in mesocosm experiments and field applications. The test strains selected in Action B1 were efficiently grown on cheap industrial media by use of the methodologies available in Actygea. The produced microorganisms were in a form and at a concentration suitable for macrocosm and large-scale applications. Furthermore, the identification of a suitable amendant for the bioremediation process was preliminarily assessed and was implemented in the following phases of the project whenever necessary.

Large scale production of microorganisms for *in situ* activities

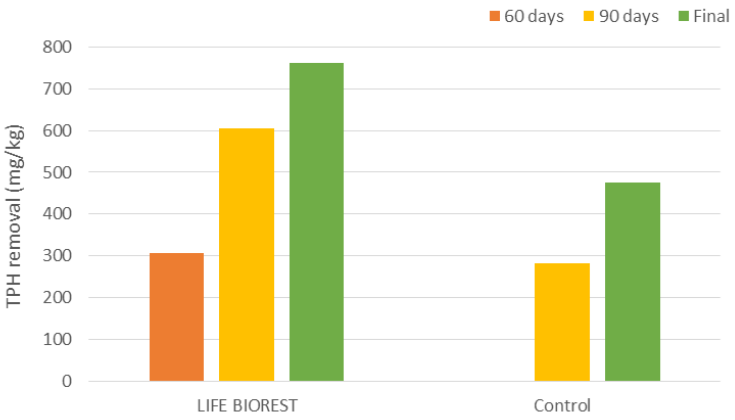
- Microbial cultures were prepared and used for the preparation of the biopile in the Fidenza testing site. A protocol for the production of suitable amounts of microorganisms for the treatment of large volumes of soil was produced.
- The microorganisms were applied successfully to the biopile and did not have any relevant impact on the regular activities of the biopile preparation. The developed methodology and the identified media were suitable for the application to any other biopile for the bioremediation of oil derived pollutants.
- Better than expected results in the growth of microorganism allowed a reduction of the fermentation volumes.
- The protocol for the production of microorganisms for the application in bioremediation was sufficient to allow the treatment of 530 tons of soil. The biopile cost was estimated at 150 €/ton (of which 110 €/m^3 are for running). The protocol was sustainable under the following aspects: the costs of production of bioaugmented bacteria were in the range of 2-20 € for each ton of soil to be treated. The materials used for production were by-products (as chipped wood and tomato seeds) from the wood and food industry.

In situ bioremediation and revegetation

In situ bioremediation

The biopile (with a total volume of 400 m^3) was prepared and treated with the best microbial consortium.
Its preparation started in February 2018 and was prepared with 530 tons of contaminated soil. The soil was then shaped in a standard 4m-height biopile. The rice husks were added to the biopile, which was continuously aerated, and the humidity of the soil was controlled all along the incubation period.

The chart here shows the removed hydrocarbons (TPH, mg/kg of soil) by the bioaugmented biopile (LIFE BIOREST) and the not-bioaugmented one (control) during time. The bioaugmentation led to faster pollutant degradation. LIFE-BIOREST biopile removed up to 300 mg/kg of hydrocarbons already after 60 days. At the end of the treatment, it was more efficient: TPH removal was 38% higher than the control. The toxicity was even halved in comparison with the control.



Soil revegetation – lab, greenhouse and field tests

- A selection was carried out to identify the plant species most performing in terms of pollutants resistance and degradation:
  Agricultural crops: wheat, barley, maize, sorghum, tomato
  Forage crops: cocksfoot, fescue, ryegrass, clover
  Biomass/Industrial crops: miscanthus, hemp, giant reed.
- Germination tests and greenhouse experiments highlighted sorghum, fescue and ryegrass as the most performing species for phytoremediation and revegetation

- Bioassays focused on the acute toxicity showed a reduction of ecotoxicity of the soil treated in the bioaugmented biopile

Plants selection and re-vegetation conditions

Two experiments were performed to identify the most suitable plants species to be used for revegetation: a germination test with polluted soil eluate and a germination test on contaminated untreated soil of Fidenza site.

14 plant species were tested in a germination assay. Among them, three species resulted the most indicated for phytoremediation in the soil contaminated with PAHs and BTEX: *Sorghum bicolor, Trifolium pratense and Festuca arundinacea*, which were then used to treat the mesocosms soil after the bioaugmentation step.

Revegetation and restoration of soil ecological functions

Due to critical aspects incurred during the project, the contingency plan considered greenhouse trials with pot experiments. At the end of the revegetation test, all planted pots showed good plants growth, with slightly higher biomass yields in the pots containing the soil taken from the treated biopile. It was also shown that the selected species led to a further reduction in contaminants concentrations.

A revegetation project on an area of 600 m^2 was developed, by planting the three plant species selected for the phytoremediation application:
Sorghum bicolor, Trifolium pratense and *Festuca arundinacea.*
The revegetation project aimed at providing a permanent demonstration area to carry out guided visits for high school students.

Mapping and identification of hydrocarbon polluted sites in Italy

- The protocols for bioremediation being implemented in LIFE-BIOREST have an excellent potential for being diffused around Europe, starting from the 3 nations studied (Italy, France and Spain). The mapping and identification of different hydrocarbon polluted sites were carried out to provide a detailed mapping of the European sites where the LIFE BIOREST protocol could be replicated.
- In the first months of 2018, an agreement on the soil samples and protocols for the method replication on Spanish and French sites was defined. The polluted soil has been inoculated with the selected microbial consortium, and control has been set up without the bioaugmentation.
- The most relevant polluted sites in Italy are classified as Sites of National Interest (Siti di Interesse Nazionale, SIN). Among the 39 SINs, the Fidenza site is where LIFE-BIOREST activities are located. In Andalusia, the total number of contaminated sites is 29,277, and a total of 4,142 sites are located in France, well distributed in the national territory.

LIFE BIOREST replicability and transferability

The approach developed in the LIFE BIOREST project is somehow going beyond the bioremediation of organic pollutants. Indeed, considering the potential of microorganisms, the same strategy could be adopted in improving soil fertility, in speeding up the shifting of soils from traditional to organic agriculture, in preventing (by ready-to-use protocols) pollution by "at risk" industrial activities. Therefore, LIFE BIOREST has the potential to integrate into several of the different policies of the EU. In conclusion, from the social point of view, contaminated soil represents a lost economic opportunity and a threat to the health and wellness of human beings and the environment.

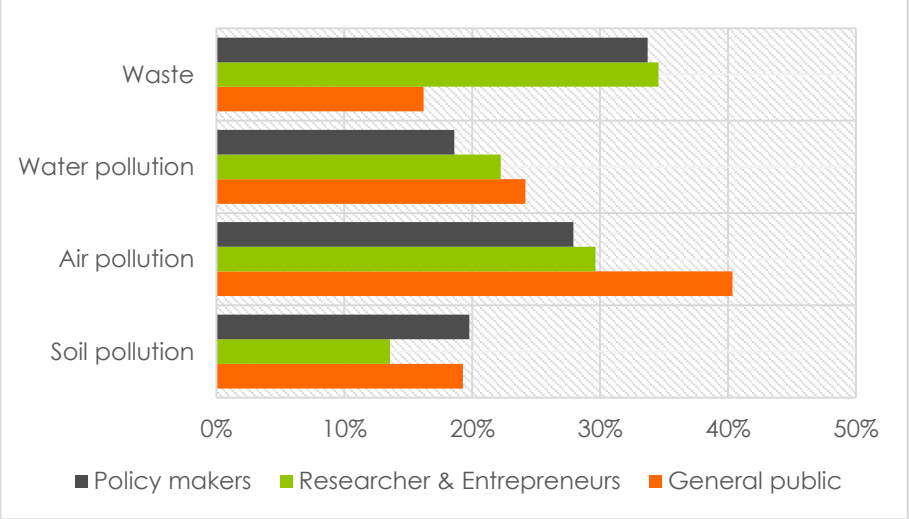
This contamination is the legacy of industrialisation and insufficient environmental legislation and enforcement. LIFE BIOREST is the legacy of an approach, which we ideally want to export worldwide. The project has demonstrated that bioremediation with bioaugmented and biostimulated microorganisms is cheap and environmentally sound. With the technologies available to date, we foresee an application of the developed method also to the deep soil which could eventually avoid removal and mechanical mixing.

Socio-economic impact study

The main socio-economic effects of LIFE BIOREST project have been identified as a **growing citizens' sensitivity to environmental emergencies** – in particular to soil pollution – and the widening of the proposed bioremediation method at national and European level.
The socio-economic study was aimed at defining the main bottlenecks to increased use of bioremediation.
The impact assessment is focused on collecting information related to:

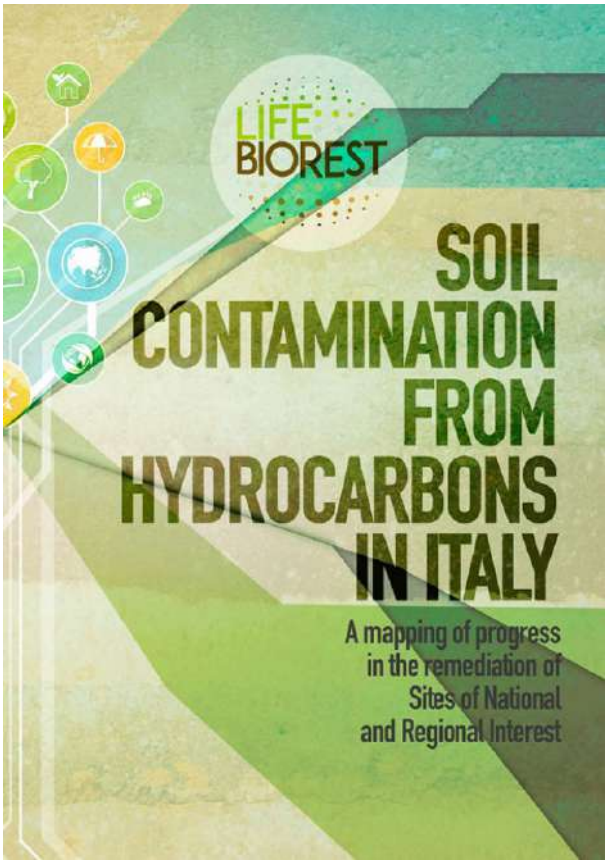
- **public initiatives promoted to improve the protection of the environment** and the quality of citizens life (*e.g.* improvement of waste management efficiency, prevention and treatment of air, water and soil pollution, reduction of soil consumption and restoration of ecosystem services, etc.)
- **priority areas of public investments** in the medium-short term (*e.g.* improvement of energy efficiency in public transport, increase in green areas and leisure facilities, etc.)
- **green Public Procurement initiatives** (GPP) adopted
- **main obstacles to the full integration of GPP solutions** in current legislative processes

During the public events and communication actions, a socio-economic impact study has been conducted by surveys handed out to the target audience.
A total of **750 surveys and 5,548 responses** were collected. The survey results suggest the need to strengthen public awareness of soil pollution. Data about the environmental emergency perception for each of the three survey target audiences (1. General Public, 2. Researchers, entrepreneurs and 3. Public authorities and policymakers audiences) are here below reported.



The second part of the Study has been focused on mapping the state of pollution and the main obstacles to the full application of the bioremediation method. A database of all the sites of regional and national interest has been developed starting from 20 registries produced by the Italian regions. The database represents the **first national instrument for the census of all polluted sites in Italy**, where they are classified by region, source and type of

contaminants with a special focus on hydrocarbon pollutants and state of implementation of reclamation. **Over 20,000 Italian sites have been mapped, of which 9,487 require remediation interventions. This category includes 2,119 sites contaminated by hydrocarbons (oil and derivatives), equal to 22% of the total number of contaminated sites in Italy.**



LIFE BIOREST publication on the mapping and identification of hydrocarbon polluted sites in Italy

Dissemination initiatives – Raising awareness

34

Public events, seminars, workshops

2,600 people

1,350

Students and researcher involved in bioremediation lessons, lab practices, International Summer School

- **1,290 students** of secondary and high schools involved in seminars and soil protection initiatives
- **30 high school teachers** engaged in bioremediation training
- **30 international researchers** involved in the International

240

People involved in networking actions with other LIFE projects

7 other LIFE projects involved in dissemination initiatives

24

Project presentations

presentations and poster exhibition at international scientific conferences

50+

Project insights, press releases, articles and scientific papers

- **15 press releases** published by LIFE BIOREST
- **1 newsletter**
- **4 articles** on the leading technical national magazines
- **6 scientific papers** on peer reviews journals + 7 under preparation

7,000

people reached via project website

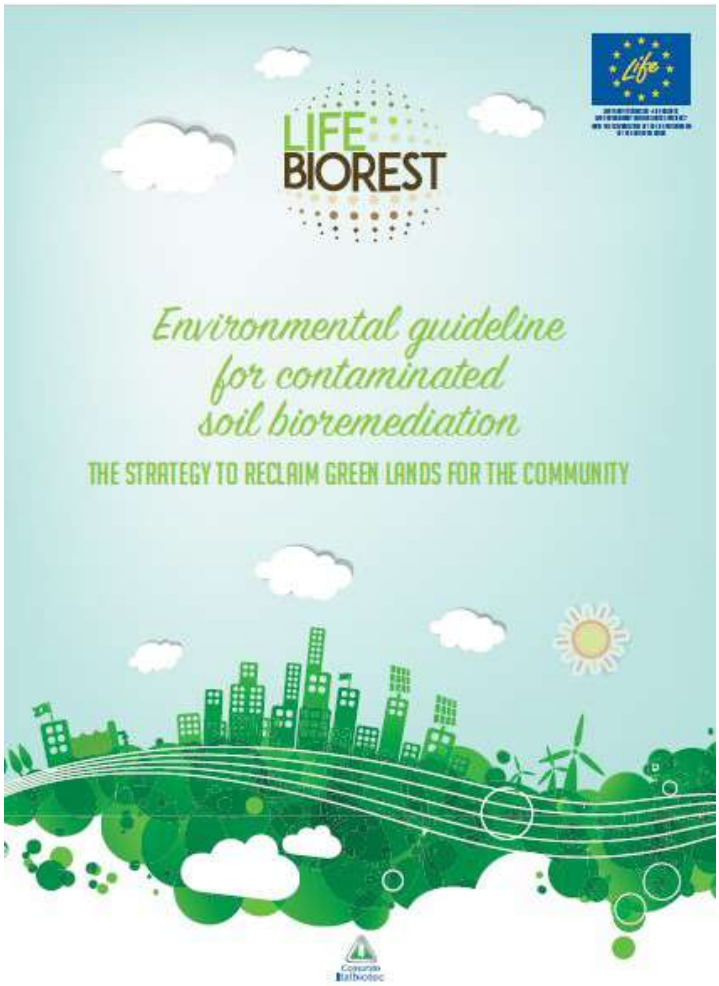
- **7,327 users reached**
- **10,227 number of sessions** (1.4 session per user)
- **24,012 number of visualisations** per page

Life Biorest ambassadors

"Ambassadors of Science. Biotechnologies are for you!" An ideas competition for soil protection (Bologna – Fondazione Golinelli, September 25, 2017)**.** The **#VerdePerTutti** ideas competition aimed at involving students in pollution issues, particularly soil, its prevention and treatment, and encouraging them to become active in raising public awareness.

1. SOIL REMEDIATION LABS EXPERIENCE. Students carried out practical laboratory activities on bioremediation method as testing at industrial scale at the LIFE BIOREST site. Over **800 students** were involved in lab activities.
2. BECOME SOIL PROTECTION AMBASSADOR. The **#VerdePerTutti competition** was designed as a video contest focused on encouraging students to become "Soil protection ambassadors". Over 200 students participated in the #VerdePerTutti contest with **50 promotional videos produced** and web disseminated to raising public awareness on soil pollution, bioremediation and sustainable use of natural resources.
3. IN-FIELD SOIL REMEDIATION ACTIVITIES. Lessons on phytoremediation at the LIFE BIOREST testing site have been carried out. They provide a shared direct experience of the biological method tested by students at the lab scale.

Bioremediation Guideline



The **"Environmental guideline for contaminated soil bioremediation – the strategy to reclaim green lands for the community"** represents the final publication of the LIFE BIOREST project, elaborated in three languages (English, Spanish and French). This publication has the objective of presenting to the local authorities the collected and elaborated recommendations and results obtained through the LIFE BIOREST project.

The LIFE BIOREST project was conceived to demonstrate the efficiency and cost-effectiveness of an innovative and sustainable solution for the bioremediation of hydrocarbon-contaminated soils with the final goal of restoring the ecological functions of soils, preventing the loss of fertility, biodiversity and resilience and reclaiming new green areas back for the community.

This document was developed to provide a model for the application of a bioremediation technique in those municipalities affected by contaminated sites, using the case-study of the Fidenza site, where the LIFE BIOREST project took place.

For this reason, **LIFE BIOREST proposes a model for the application of the bioremediation treatment to the contaminated sites, with the potential for it to be diffused around Europe.**

Impact on policy instruments

➢ MAPPING OF HYDROCARBON CONTAMINATION

The Italian legislation on contaminated sites (article 251 of Legislative Decree 152/06) established the regional registries to identify sites subjected to environmental reclamation. A state of the extreme fragmentation of data collection hinders a program for national restoration and the evaluation of the most efficient remediation methods. The LIFE BIOREST mapping of polluted hydrocarbon soil can support regions on the evaluation of the state of contamination

➢ ENVIRONMENTAL IMPACT ASSESSMENT OF BIOREMEDIATION METHOD

The LIFE BIOREST contributes to the evaluation of the economic and ecological sustainability of the bioremediation and supports its potential application in the SINs that present hydrocarbon pollution.

➢ SOIL THEMATIC STRATEGY (COM(2006)231)

The LIFE BIOREST supports the second objective, which is focused on restoring degraded soils to a level of functionality consistent with intended use, also considering the cost implications of the restoration of soil. The project develops a sound solution for the restoration in situ of contaminated soils by oil pollutants through bioremediation approach, but it also addresses the socio-economic aspects of the remediation actions by proving their cost-effectiveness and scalability.

➢ EU BIODIVERSITY STRATEGY FOR 2020 (COM(2011) 244 FINAL)

The LIFE BIOREST supports the Target 2 related to the restoration of the degraded ecosystems, by providing effective solutions for the in-situ bioremediation of contaminated soils by oil pollutants, hence providing for the necessary conditions for enhancing biodiversity and restoring ecosystem functions.

➢ SEVENTH ENVIRONMENT ACTION PROGRAMME (7TH EA")

The LIFE BIOREST support the priority objectives of the 7th EAP to:

- safeguarding the Union's citizens from environment-related pressure and risks to health and well-being, by providing a feasible solution for the issue of soils heavily contaminated by toxic compounds
- turning the Union into a resource-efficient, green and competitive economy, by proposing a cost-effective working protocol to bioremediate soils that valorize waste biomass.